



SPI-PON 812R Kit

Use Instructions

CAUTION: Thoroughly read all instructions and warnings on container labels prior to use of this kit. SPI-PON 812R is our replacement for SPI-PON 812 embedding resin (effective July 2024)

SPI-PON 812R resin can be used in various embedding applications in both plant, animal and material sciences. A cured resin block of required hardness, to match the embedded material, can be made by varying the proportions of DDSA (softener) and NMA (hardener). It is well known that hard specimens in a soft resin block will tear away from the block, whereas soft specimen materials will section unevenly if embedded in a hard resin formulation. In addition to the wide range of hardness that is attainable, other advantages include improved penetration (in biological tissue), contrast, and ease of obtaining stable thin sections. The same properties offered in SPI-PON 812 are also offered in SPI-PON812R such as: rapid penetration, greater contrast, easy sectioning, stability under the electron beam satisfactory staining of most thick section for LM and thin section for EM.

Recommended Formulations and Mixing Instructions (we offer 2 options)

Option#1:

	<u>Small Amount</u>	<u>Medium Amount</u>	<u>Large Amount</u>
MIXTURE A:			
SPI-PON 812R	5 ml	20 ml	62 ml
DDSA	8 ml	31 ml	100 ml
MIXTURE B:			
SPI-PON812R	8 ml	20 ml	100 ml
NMA	7 ml	17 ml	90 ml

FINAL EMBEDDING MIXTURE:

MIXTURE A:	13 ml	51 ml	162 ml
MIXTURE B:	15 ml	37 ml	190 ml
DMP-30*	0.42-0.56 ml	1.3-1.7 ml	5.3-7.0 ml

*For better penetration and stability, BDMA is recommended in place of DMP-30. The quantity of BDMA which is required is 2.5-3.0% while DMP-30 is 1.5-2.0%.

Option#2: (This is a simpler option and uses a one-step single mix formula. The following formulations may be used depending on the desired hardness of the block)

	<u>SOFT</u>	<u>MEDIUM</u>	<u>HARD</u>
SPI-PON 812R	20 ml	20 ml	20 ml
DDSA	22 ml	16 ml	9 ml
NMA	5 ml	8 ml	12 ml
DMP-30	0.70-0.94 ml	0.66-0.88 ml	0.62-0.82 ml
(IF USING BDMA INSTEAD of DMP-30):	1.18-1.4 ml	1.1-1.3 ml	1.0-1.2ml

NOTE- you can use DMP-30 or BDMA, but you cannot use both when making the formulations above.

Slight variations of the accelerator (DMP-30 or BDMA) will drastically affect the color and brittleness of the block. Thorough mixing is imperative to be able to achieve uniform blocks.

While preparing SPI-PON 812R, the hardness of the block can be varied to suit various sectioning conditions depending on the ratio of Mixture A and Mixture B in the final embedding mixture. An increase in the proportion of Mixture B will make the block harder. A mixture of 1:1 has proven most successful for general use. All components of the kit should be kept at room temperature.

Although the mixture can be stored for up to 6 months at 4°C it is highly recommended that freshly prepared embedding medium always be used.

EMBEDDING:

For best results use SPI Supplies or BEEM embedding molds and capsules. The following part# have given the best results:

02330-MB - BEEM 1001B Capsules, Size 3, pack of 100

02441M-AB – Silicone Embedding Mold, Maroon, 24+3 Flat (27 Cavities), Chemical & Heat Resistant

For additional mold and capsules, visit our website at www.2spi.com

Transfer each sample to a dry capsule or mold and fill with the embedding medium. Cure the medium in an oven at 60°C for 24 hours. Blocks can be trimmed and sectioned after returning to room temperature.

MIXING OF RESIN COMPONENTS

The preparation and mixing of the resin components must be thorough and should always be performed in a fume hood and/or a covered vessel and for a period of at least 5-10 minutes. After the DMP-30 has been accurately measured and completely mixed into the selected resin, the complete formulation can be used to impregnate and/or embed the sample material. Avoid contact of the mixture with moisture. The mixture, without DMP-30 is stable for 6 months at 4°C, or for several days at room temperature. When refrigerated, the mixture, in its closed container must be allowed to equilibrate to room temperature before use, to prevent condensation of moisture.

SAMPLE PREPARATION

Sample for resin infiltration and embedding should be dry. Biological samples, fixed and dehydrated through absolute alcohol are further dehydrated in two 15 minute changes of propylene oxide. Infiltration is accomplished using the complete formulation and propylene oxide (1:1), while gently agitating for at least 1 hour. Infiltration of the specimen is continued in at least one change of the complete formulation for 1 hour. Final embedding of the sample is accomplished by placing the specimen in aliquot of the complete formulation, located in appropriate flat or capsular embedding molds. Hardening or polymerization of the above resins can be accomplished following curing in an oven at 60°C for 24-48 hours, after which time the blocks are allowed to cool to room temperature for subsequent ultramicrotomy.

REFERENCES

1. Luft, J.H.J. *Biolphs. Biochem, Cytol.* 9, 409 (1961)

DISCLAIMER

The information given in this bulletin is to the best of our knowledge accurate, but no warranty is expressed or implied. It is the user's responsibility to determine the suitability for his own use of the products described herein; and since conditions of use are beyond our control, we disclaim all liability with respect to the use of any material supplied by us. Nothing contained herein shall be construed as a recommendation to use any product or to practice any process in violation of any law or any government regulations.